Bacterial Transformation

Introduction

Basic protocol for bacterial transformation from addgene (https://www.addgene.org/plasmid-protocols/bacterial-transformation/).

Materials

- Competent cells
- DNA to transform
- LB medium
- Appropriate antibiotics
- Agar plates containing appropriate antibiotics

Procedure

Transformation

1. Take competent cells out of -80°C and thaw on ice (approximately 20-30min).
2. Take agar plates (containing the appropriate antibiotic) out of 4°C to warm up to room temperature or place in 37°C incubator.
3. Mix 1 to 5µl of DNA (usually 10pg to 100ng) into 20-50µL of competent cells in a microcentrifuge or falcon tube. GENTLY mix by flicking the bottom of the tube with your finger a few times.
4. Place the competent cell/DNA mixture on ice for 20-30min.
5. Heat shock each transformation tube by placing the bottom 1/2 to 2/3 of the tube into a 42°C water bath for 30-60 seconds (45sec is usually ideal, but this varies depending on the competent cells you are using).
6. Put the tubes back on ice for 2 min.
7. Add 250-500µl LB or SOC media (without antibiotic) and grow in 37°C shaking incubator for 45min.
8. Plate some or all of the transformation onto a 10cm LB agar plate containing the appropriate antibiotic. We recommend that you plate 50µL on one plate and the rest on a second plate. This gives the best chance of getting single colonies, while allowing you to recover all transformants. You can spread the transformation on even more plates, and plate the rest of the transformation the next day.
9. Incubate plates at 37°C overnight.